Day 39-Monday-7/28/14

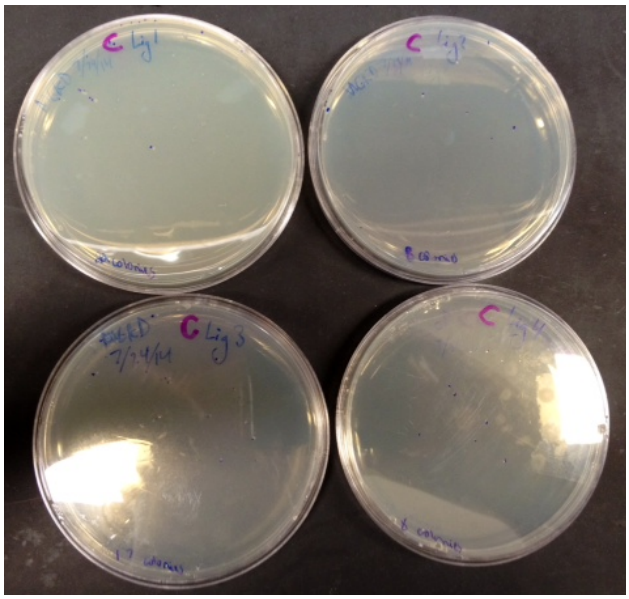
- Alex and Chloe grew liquid cultures of 17K and INP clones (9C)

- Kayla and Mike checked the 32C sequence results. Unfortunately, the plasmid that was sent for sequencing only contained the RBS, ATF1 gene, and the double terminator but not the constitutive promoter. Thus, it was decided that a mass-miniprep would be the most effective approach. 20 liquid cultures of 32C were prepared in an attempt to find the correct construct. In addition, we observed the transformed pARS plates from last Thursday, and noted colony growth on every plate (including the control). The table of colony counts can be seen below. Colony growth was relatively low (<30), so we decided to leave the plates in the warm room until needed for the liquid culture, a picture of the plates can also be seen below. 10 liquid cultures were prepared using colonies on plate 3.

  Colony Counts

Plate	Colonies
1	11
2	8
3	17
4 (control)	8

  26C Plate Pictures



Based on the results from the OD agglutination quantification, we have decided that the process is unfeasible. It appears that any forces capable of directing fluid through the filters also tears any clumped cells apart. Simply put, the bonding interactions of the antibodies are not sufficient to withstand a significant force applied to them. They are only strong enough to provide visual clarification of agglutination. Although we have abandoned this protocol, we still have a data modeling segment in the form of the GC/MS enzyme efficiency of the ATF1 gene.

- Corbyn and Shawna started the day by analyzing the sequence of $29A_2$ (Promoter+RBS+BCLA+CAEV) and $30A_{3,5,6}$ (Promoter+RBS+BCLA+YFP) to find that they were all correct. We also moved our 31C plates that had been left benchtop over the weekend to the warm room to allow the colonies to grow. The colony counts can be seen in the table below, with a labeled picture.

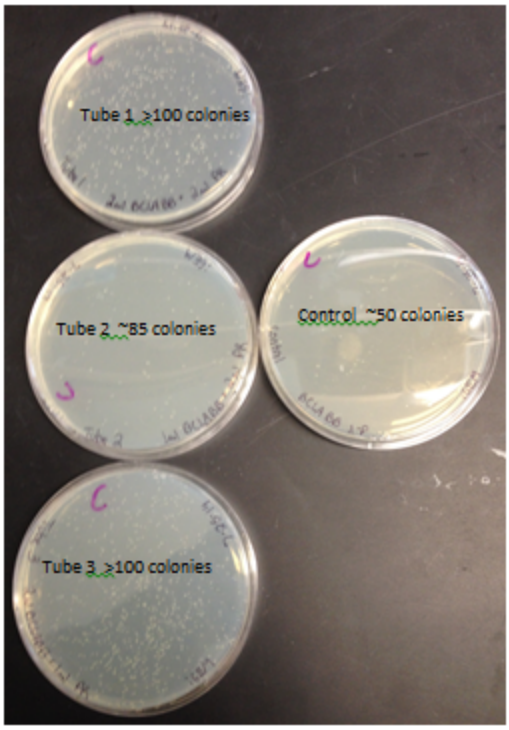
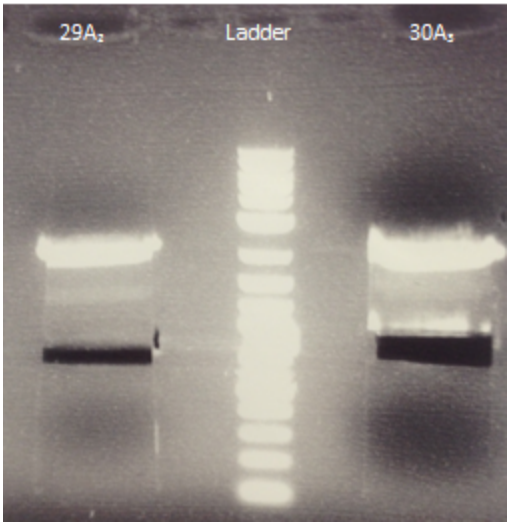


Plate	Colony Count
Tube 1: 2uL BCLA BB+ 2uL PR	>100
Tube 2: 1uL BCLA BB+ 2uL PR	~85
Tube 3: 2uL BCLA BB+ 1uL PR	>100
Control: BCLA BB3 X-P	~50

Then, we created 50 uL digests of $29A_2$ and $30A_3$ with E-S as well as an E-X digest of 1C (double terminator). After allowing them to digest for about an hour, we CIPed $29A_2$ and $30A_3$ and then ran them on a 1% gel with two larger wells for about an hour for gel purification. A picture of the gel after the pieces had been cut out for gel purification can be seen below.



We also heat killed the E-X digestion of 1C. Once the gel purification was complete, we set up ligations of the 1C to both $29A_2$ and $30A_3$. These ligations have been renamed 33C and 34 C, respectively. The ligations we performed can be seen in the table below, and we only did 5 instead of the usual 6 because we ran out of DNA ligase. Once these ligations were complete, we put them in the 20degree freezer overnight because we ran out of time for a transformation.

Tube Label	uL of $29A_2$	uL of $30A_3$	uL of 1C
1-1	2	0	2
1-2	2	0	1
2-1	0	2	2
2-2	0	2	1
2-3	0	1	2

At the end of the day, we also liquid cultured 6 colonies from the 31C plate 3 as well as one colony from the $30A_5$ glycerol stock (Promoter+RBS+BCLA+YFP) with the intention of using it for microscopy the next day.

Day40 - Tuesday 7/29/14

- A large number of minipreps were made, distributed among the three groups, this includes 20 minipreps of 32C and 10 minipreps of 26C. The concentrations and preparers are in the table below:

32C minipreps

Tube	Concentration (ug/uL)	Made by

1	0.10	Shawna & Corbyn
2	0.19	Shawna & Corbyn
3	0.11	Shawna & Corbyn
4	0.07	Shawna & Corbyn
5	0.10	Shawna & Corbyn
6	0.16	Shawna & Corbyn
7	0.08	Alex & Chloe
8	0.17	Alex & Chloe
9	0.13	Alex & Chloe
10	0.18	Alex & Chloe
11	0.04	Alex & Chloe
12	0.10	Alex & Chloe
13	Didn't grow	N/A
14	0.16	Alex & Chloe
15	0.16	Alex & Chloe
16	0.14	Alex & Chloe
17	0.31	Alex & Chloe
18	0.16	Alex & Chloe
19	0.224	Kayla and Mike
20	0.245	Kayla and Mike

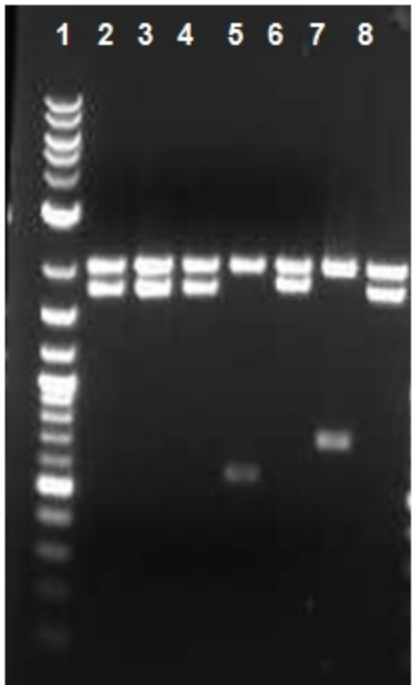
<u>26C Minipreps</u>

Tube	**Concentration (ug/uL)**	**Made by**
1	0.208	Kayla and Mike
2	0.117	Kayla and Mike

3	0.233	Kayla and Mike
4	0.296	Kayla and Mike
5	0.365	Kayla and Mike
6	0.369	Kayla and Mike
7	0.361	Kayla and Mike
8	0.192	Kayla and Mike
9	0.218	Kayla and Mike
10	0.172	Kayla and Mike

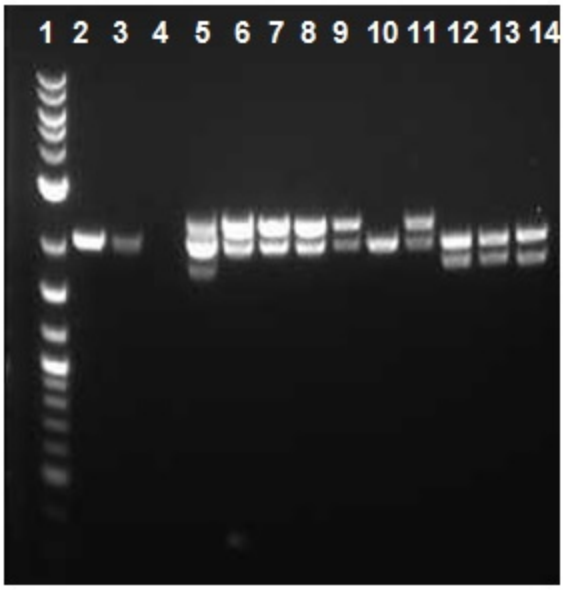
After all of the minipreps were made, 30 test digests were set up. All minipreps were digested with EcoRI and PstI for one hour at 37ºC and then run on 3 gels. A digest of the RBS/ATF1/DT construct was also run on the gel as a control. All of the gels can be seen below. Based on the gels, the 32C minipreps 1,2,3,5,19, and 20 potentially have the correct insert. Minipreps 6,7,8, and 10 for 26C also appear to have the correct insert. All of these samples will be sent off for sequencing tomorrow.

Gel 1: 32C Minipreps 1-6



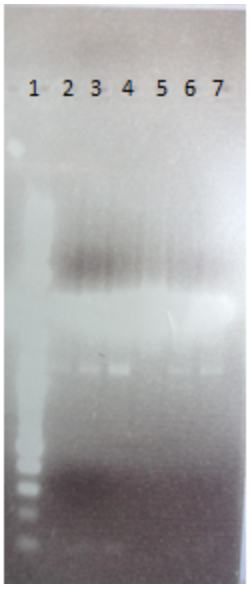
Lane	Sample
1	2-log ladder
2	32C Miniprep 1
3	32C Miniprep 2
4	32C Miniprep 3
5	32C Miniprep 4
6	32C Miniprep 5
7	32C Miniprep 6
8	Control

Gel 2: 26C Minipreps 1-10 and 32C Minipreps 19 and 20



Lane	Sample
1	2-log ladder
2	26C Miniprep 1
3	26C Miniprep 2
4	26C Miniprep 3
5	26C Miniprep 4
6	26C Miniprep 5
7	26C Miniprep 6
8	26C Miniprep 7
9	26C Miniprep 8
10	26C Miniprep 9
11	26C Miniprep 10
12	32C Miniprep 19
13	32C Miniprep 20
14	Control

- Shawna and Corbyn started the day by doing 12 minipreps. The first 6 of these were liquid cultures of 32C prepared by Mike and Kayla the day before. The second 6 were liquid cultures of 31C (Promoter+RBS+BCLA) that we prepared the day before. These minipreps were then test digested using E and P. A gel photo of our samples (31C)with labeled lanes can be seen below.



Lane	Contents
1	Ladder
2	$31C_1$ E-P digest
3	$31C_2$ E-P digest
4	$31C_3$E-P digest
5	$31C_4$ E-P digest
6	$31C_5$ E-P digest
7	$31C_6$ E-P digest

It was determined that none of the 31C minipreps had the correct insert. We realized that the digestions used in an attempt to create this part were done with the wrong enzyme pairs. Using the 33C and 34C ligations from the day before, Shawna and Corbyn performed transformations of what should be Promoter-RBS-BCLA-CAEV-DT and Promoter-RBS-BCLA-YFP-DT. There were five plates in total. Three were different ligations of YFP and two were different ligations of CAEV. In the afternoon, Shawna and Corbyn set up overnight digestions of BCLA BB3and of

$28A_4$. The $28A_4$ digestion was 50uL and done with E and S because it will be gel-purified the next day, the BCLA digestion was 20uL and done with E and X.The digestions were left in the 37degree bath overnight

Day 41-Wednesday-7/29/14

- Alex and Chloe prepared gels for a western blot to be conducted tomorrow and set up an agglutination assay with GFP antibodies for 30A (BCLA+YFP), 17K (Interlab GFP biobrick), 25C (BCLA+CAEV, for control) and 9C (INP+YFP) in order to compare the agglutination efficiency of BCLA vs INP and to make sure that YFP is being expressed on the surface of 30A through BCLA by comparing it to 17K, which in theory should not agglutinate. The well plate setup is shown in the following table. All units are in ul, and every dilution from 1:50 to the final concentration of 1:25600 was performed via serial dilution beginning at the 1:50 concentrations resulting in 50 ul in each well.

	1:2	1:10	1:50	1:100
BclA-CAEV	12.5 BSA 12.5 GFP-G1 Antibody	22.5 BSA 2.5 GFP-G1 Antibody	99 BSA 2 GFP-G1 Antibody	50 BSA 50 From previous well
GFP	12.5 BSA 12.5 GFP-G1 Antibody	22.5 BSA 2.5 GFP-G1 Antibody	99 BSA 2 GFP-G1 Antibody	50 BSA 50 From previous well
INP-GFP	12.5 BSA 12.5 GFP-G1 Antibody	22.5 BSA 2.5 GFP-G1 Antibody	99 BSA 2 GFP-G1 Antibody	50 BSA 50 From previous well
BclA-GFP	12.5 BSA 12.5 GFP-G1 Antibody	22.5 BSA 2.5 GFP-G1 Antibody	99 BSA 2 GFP-G1 Antibody	50 BSA 50 From previous well
BclA-CAEV	12.5 BSA 12.5 GFP-8H11 Antibody	22.5 BSA 2.5 GFP-8H11 Antibody	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well
GFP	12.5 BSA 12.5 GFP-8H11 Antibody	22.5 BSA 2.5 GFP-8H11 Antibody	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well
INP-GFP	12.5 BSA 12.5 GFP-8H11 Antibody	22.5 BSA 2.5 GFP-8H11 Antibody	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well
BclA-GFP	12.5 BSA 12.5 GFP-8H11 Antibody	22.5 BSA 2.5 GFP-8H11 Antibody	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well

- Kayla and Mike started off by performing a surface protein detection trial with three different cell strains under two sets of conditions:

Fixed Cells: 20ul culture on slide, dried in hood ~10 minutes, Fixed in MeOH 10min, rinsed in PBS for 3min, blotted dry, 10 ul 50%glycerol/PBS and covered with a coverslip.

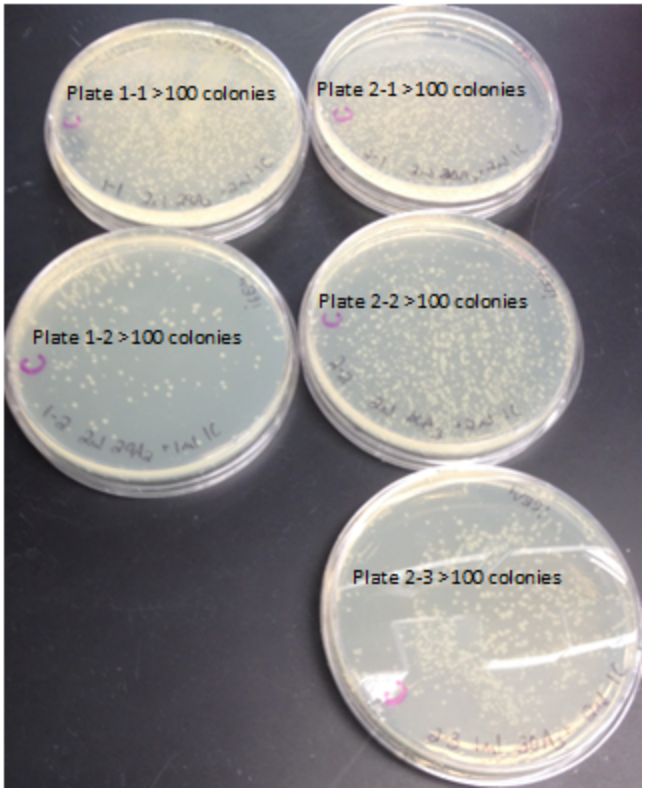
Live Cells: 10ul of culture, and covered with a coverslip

Surface Protein Specimens

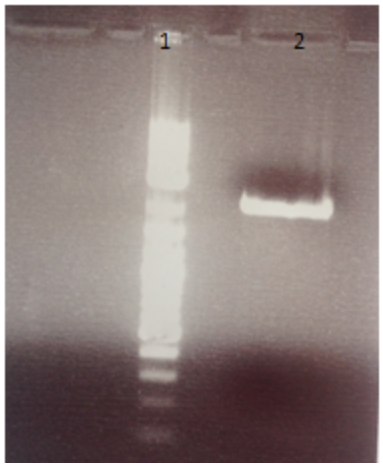
Slide	Specimen	Observations (5x-40xOil)
I	Fixed 9C	Few/no bacteria, some clumpings of glowing material
II	Fixed 30A	under 40xOil with Apotome slicings, a number of cells appear to express darker pigmentation in their centers, suggesting a surface protein Corbyn and expression.
III	Fixed 17K	
1	Live 9C	Unsuitable for slicings, too mobile
2	Live 30A	Unsuitable for slicings, too mobile
3	Live 17K	Unsuitable for slicings, too mobile

Kayla and Mike also sent off 6 samples for DNA sequencing (VF2 primer), incuding 26cmin7, 26cmin8, 26cmin10, 32Cmin19, 32Cmin5, and 32cmin20.

- Corbyn and Shawna first collected the plates of 33C and 34C that were in the warm room overnight. A picture of the plates with colony counts can be seen below, each plate was over 100 colonies.



Then, we heat killed the BLCA BB3 that had been digesting overnight and CIPd and gel purified $28A_4$. Unfortunately, the gel purification was unsuccessful because there was not enough of the insert's DNA. A picture of the gel with labeled lanes can be seen below, and you can see that a band for $28A_4$, which should be around 100 BP is not visible.



Lane	Contents
1	Ladder
2	$28A_4$ gel purification

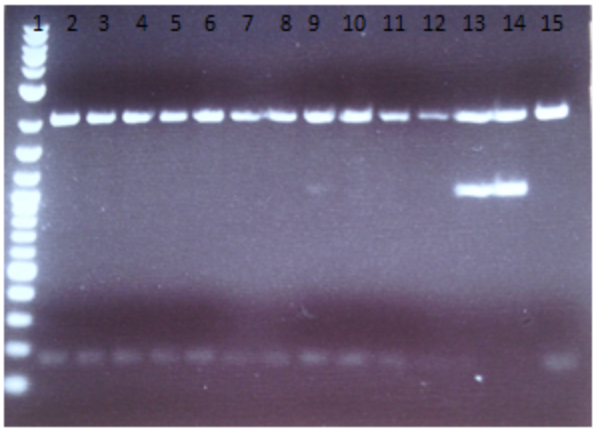
Due to the failed gel purification, we digested $28A_4$ once again, this time performing two digestions, one using the normal 2uL of DNA and another using 4uL of DNA. We let these digestions run for about 2 hours and then heat killed. Simultaneously, we CIPd BCLA BB3 and heat killed. Once both digestions were heat killed, we set up 6 ligations. A table of the tube labels and their contents can be seen below. We did not have time to transform so we decided to save the ligation in the -20 overnight. Once transformed, this ligation will be renamed 25C.

Tube Label	uL of $28A_4$ digestion 1	uL of $28A_4$ digestion 2	uL BCLA BB3
1	2	0	2
2	2	0	1
3	1	0	2
A	0	2	2
B	0	2	1
C	0	1	2

At the end of the day, we liquid cultured 2 colonies from 33C and 6 colonies from 34C.

Day 42-Thursday, 7/31/14

- Kayla and Mike checked the sequencing results for the 26C and 32C minipreps. All minipreps that were sent (26C minipreps 7,8,10 and 32C minipreps 5,19,20) had the correct promoter sequence, so three liquid cultures were prepared. Two of the liquid cultures were made from the 26C glycerol stock. One had just 5mM isoamyl alcohol added to it while the other had 5mM isoamyl alcohol and 25uM arsenite added to it. The other liquid culture was prepared from a 32C glycerol stock. It contained only 5mM isoamyl alcohol. Kayla also worked on the Power Point presentation for the Science Project and Equipment Camp at Wachusett Regional High School.
- Alex and Chloe ran the western blot, extracted the proteins onto a membrane, and stored it in buffer for further development on Monday. Another agglutination assay was prepared with the same samples as before, but this time using the CAEV antibody.The well plate setup is shown in the following table. All units are in ul, and every dilution from 1:50 to the final concentration of 1:25600 was performed via serial dilution beginning at the 1:50 concentrations resulting in 50 ul in each well.
- Shawna and Corbyn started the day by miniprepping the 12 liquid cultures of 33C and 34C.The minipreps were then nanodropped, and we performed a test digest on them using E-P. We then ran the digests on a 1% gel for about an hour, and a picture of the gel with labeled lanes can be seen below.



Lane	Contents
1	Ladder
2	$33C_1$
3	$33C_2$
4	$33C_3$
5	$33C_4$
6	$33C_5$
7	$33C_6$
8	$33C_6$
9	$34C_1$
10	$34C_2$
11	$34C_3$
12	$34C_4$
13	$34C_5$
14	$34C_5$
15	$34C_6$

As you can see, none of the 33C clones had a band at the correct size (~1000), however $34C_5$ has the correct sized bands. We then transformed the 25C ligations that were kept in the freezer overnight.

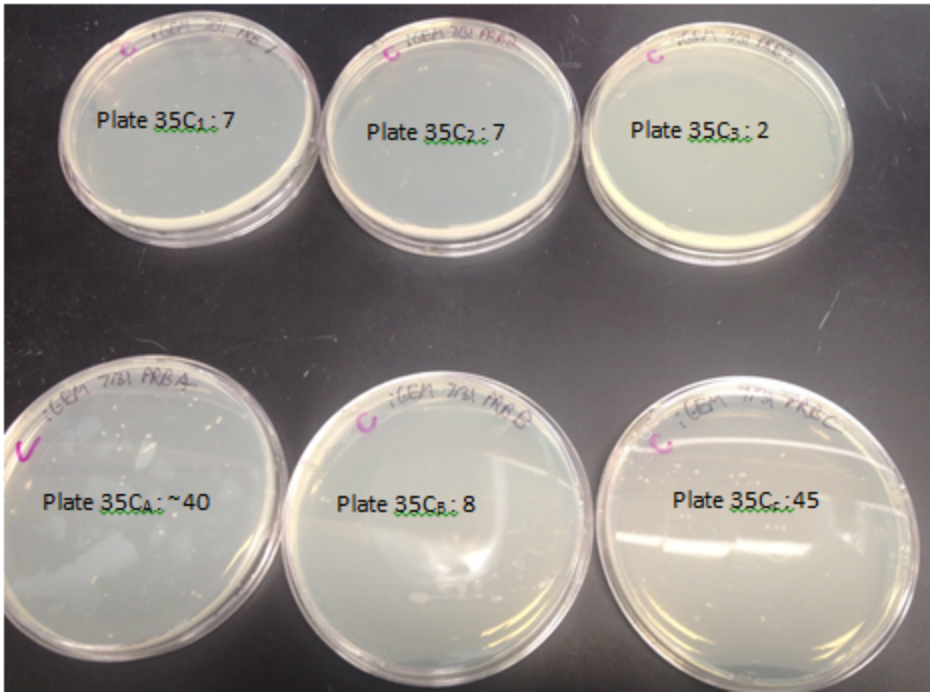
Day 42-Friday, 8/1/14

- Kayla and Mike checked the overnight liquid cultures. Unfortunately, no bacteria gre because the shaker had been turned off at some point in the night. The shaker was turned on again and the cultures were checked at the end of the day. Only the 26C culture with isoamyl alcohol had grown. Kayla also finalized the presentation for the camp at Wachusett and sent a preliminary email to Erika Olson, the Director of Operations for the New England Farmers Union.

	1:2	1:10	1:50	1:100
BclA-CAEV	12.5 BSA 12.5 CAEV Antibody	22.5 BSA 2.5 CAEV Antibody	99 BSA 2 CAEV Antibody	50 BSA 50 From previous well
GFP	12.5 BSA 12.5 CAEV Antibody	22.5 BSA 2.5 CAEV Antibody	99 BSA 2 CAEV Antibody	50 BSA 50 From previous well
INP-GFP	12.5 BSA 12.5 CAEV Antibody	22.5 BSA 2.5 CAEV Antibody	99 BSA 2 CAEV Antibody	50 BSA 50 From previous well
BclA-GFP	12.5 BSA 12.5 CAEV Antibody	22.5 BSA 2.5 CAEV Antibody	99 BSA 2 CAEV Antibody	50 BSA 50 From previous well
BclA-CAEV	12.5 BSA 12.5 GFP-8H11 Antibody	22.5 BSA 2.5 GFP-8H11 Antibody	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well
GFP	12.5 BSA 12.5 GFP-8H11 Antibody	22.5 BSA 2.5 GFP-8H11 Antibody	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well
INP-GFP	12.5 BSA 12.5 GFP-8H11 Antibody	22.5 BSA 2.5 GFP-8H11 Antibody	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well
BclA-GFP	12.5 BSA 12.5 GFP-8H11 Antibody	22.5 BSA 2.5 GFP-8H11 Antibody	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well

The agglutination assay that had been left to sit overnight was checked and had turned out to be a great success. 9C (INP+YFP) and 30A (BCLA+YFP) were the only samples that agglutinated, while 25C (BCLA+CAEV, no GFP present) and 17K (GFP construct, no surface expression) did not agglutinate, proving conclusively that BCLA is expressing YFP on the surface of the cell. This along with the microscopy and the western blot will provide the main background for our experiment and proof that our construct works properly. Many pictures were taken that will be sorted through and uploaded tonight.

- Corbyn and Shawna started the day by collecting the plates of 35C (P+RBS+BCLA). A picture of the plates as well as the colony counts can be seen below.



We also sent the $34C_5$ clone for sequencing, both forward and backward, with the hopes it will come back as a Promoter+RBS+BCLA+YFP+DT.